

DOCKET NO: 278162US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :
SYUNICHI AKIBA, ET AL. : EXAMINER: HOFFMAN, SUSAN
COE
SERIAL NO: 10/550,858 :
FILED: SEPTEMBER 26, 2005 : GROUP ART UNIT: 1655
FOR: DEODORANT AGENT :

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Shunichi Akiba

Now comes _____ who deposes and declares that:

1. I am a graduate of *Tsukuba* University and received my *Ph.D.* degree in the year *1999*.

2. I have been employed by the *Kao Corporation* for the past *24* years, as a researcher in the field of *Biological Science*.

3. I am a named inventor of the above-identified application.

4. The following experiments were conducted by me or under my direct supervision and control.

Extracts of *Ginkgo biloba L.* and *Phellodendron amurense Ruprecht* were each prepared by extraction of 10 g of ginkgo leaves or phellodendron bark with 100 mL of solvent at room temperature for 7 days, followed by filtration to obtain an extract.

Application No. 10/550,858

	Solvent	Yield (mL)	Evaporation Residue (w/v)%
Ginkgo Extract A	Purified water	85	2.32
Ginkgo Extract B	50 v/v% aqueous ethanol	85	2.66
Ginkgo Extract C	95 v/v% aqueous ethanol	85	1.59
Phellodendron Extract A	Purified water	85	1.19
Phellodendron Extract B	50 v/v% aqueous ethanol	85	1.20
Phellodendron Extract C	95 v/v% aqueous ethanol	85	0.72

The extracts were tested for relative papain inhibition activity.

Inhibitory effects of the plant extracts on papain activity were measured according to the method described in *Barret et al.*, Methods in Enzymology, vol 80, p. 771, 1981.

A solution of 10 mM benzoil-L-arginine-4-nitoranilide in dimethylsulfoxide was used as a substrate for papain in this assay. A phosphate buffer containing 2 mM EDTA was added with DTT so as to be at a final concentration of DTT of 8 mM to obtain a reaction buffer.

Papain was dissolved in a buffer not containing DTT at a concentration of 1 mg/mL to obtain a papain buffer. A 5% trichloroacetic acid solution was employed for stopping reaction. Comparative experiments were conducted as follows. A tube for the measurement was charged with the reaction buffer (0.5 mL), the papain buffer (0.05 mL), the plant extracts or the commercially available plant extracts and the balance of distilled water so as to be in a total volume of 1 mL. The resultant mixture was incubated at 25°C for 5 minutes, and then added with the substrate solution (0.05 mL), followed by stirring.

After reaction at 25°C for 15 minutes, the 5% trichloroacetic acid solution (0.05 mL) was added to the thus obtained mixture. If precipitation was observed, the mixture was centrifuged at 10,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 405 nm.

Application No. 10/550,858

Relative papain inhibitory activity (%) = $(AO - AI) / AO \times 100$

AO: (absorbance of inhibitor-free mixture-absorbance of inhibitor-free blank mixture)

AI: (absorbance of sample mixture-absorbance of blank mixture)

A mixture using the purified water, and the 50v/v% aqueous ethanol solution or the 95 v/v% aqueous ethanol solution in place of the plant extracts is referred to as an inhibitor-free mixture.

A mixture using the purified water, and the 50v/v% aqueous ethanol solution or the 95 v/v% aqueous ethanol solution in place of the plant extracts, and adding the 5% trichloroacetic acid solution before adding the substrate solution is referred to as an inhibitor-free blank mixture.

A mixture using any one of the plant extracts is referred to as a sample mixture.

A mixture using any one of the plant extracts and adding a 5% trichloroacetic acid solution before adding the substrate solution is referred to as a blank mixture.

The results are shown in the table below. It is found that the plant extracts extracted with the 95v/v% aqueous ethanol solution exhibited the highest inhibitory activities.

Application No. 10/550,858

Plant Extract	Relative papain inhibitory activity (%)	
	Concentration of extract (5%)	Concentration of extract (10%)
Ginkgo Extract A	0	Not determined
Ginkgo Extract B	19.0	Not determined
Ginkgo Extract C	67.0	100
Commercially available Ginkgo Extract (produced by Mauruzen Pharmaceutical Co., Ltd)	14.4	47.7
Phellodendron Extract A	33.0	Not determined
Phellodendron Extract B	44.9	26.8
Phellodendron Extract C	62.3	87.3
Commercially available Phellodendron (Produced by Ichimaru Pharcos Co., Ltd.)	17.4	43.1

I declare under penalty of perjury under the laws of the United States of America
that the foregoing is believed to be true and correct.

Shunichi Akiba

Date 24/07/2008